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(54) Title: MEMBER OF THE HEMATOPOIETIN RECEPTOR SUPERFAMILY

(57) Abstract

Polynucleotides encoding the U4 hematopoietin receptor superfamily chain and fragments thereof are disclosed. U4 proteins and methods for their production are also disclosed.

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MEMBER OF THE HEMATOPOIETIN RECEPTOR SUPERFAMILY

Field of the Invention

The present invention relates to new members of the mammalian hematopoietin superfamily of proteins (including without limitation human and murine receptor proteins), fragments thereof and recombinant polynucleotides and cells useful for expressing such proteins.

Background of the Invention

A variety of regulatory molecules, known as hematopoietins, have been identified which are involved in the development and proliferation of the various populations of hematopoietic or blood cells. Most hematopoietins exhibits certain biological activities by interacting with a receptor on the surface of target cells. Cytokine receptors are commonly composed of one, two or three chains. Many cytokine receptors and some cytokines, such as IL-12 p40, are members of the hematopoietin receptor superfamily of proteins. Identification of new members of the hematopoietin receptor superfamily can be useful in regulation of hematopoiesis, in regulation of immune responses and in identification of other members of the hematopoietin superfamily, including cytokines and receptors.

It would be desirable to identify and determine the DNA and protein sequence for heretofore unknown members of the hematopoietin receptor superfamily.

Summary of the Invention

In accordance with the present invention, polynucleotides encoding the U4 hematopoietin receptor superfamily chain are disclosed, including without limitation those from the murine and human sources. In certain embodiments, the invention provides an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence of SEQ ID NO:4 from nucleotide 242 to nucleotide 1396;

(b) the nucleotide sequence of SEQ ID NO:6 from nucleotide 71 to nucleotide 1225;

(c) a nucleotide sequence varying from the sequence of the nucleotide sequence specified in (a) or (b) as a result of degeneracy of the genetic code;

(d) a nucleotide sequence capable of hybridizing under stringent conditions to the nucleotide specified in (a) or (b);

(e) a nucleotide sequence encoding a species homologue of the sequence specified in (a) or (b); and

(f) an allelic variant of the nucleotide sequence specified in (a) or (b). Preferably, the nucleotide sequence encodes a protein having a biological activity of the U4 hematopoietin receptor superfamily chain. The nucleotide sequence may be operably linked to an expression control sequence. In preferred embodiments, the polynucleotide comprises the nucleotide sequence of SEQ ID NO:4 from nucleotide 242 to nucleotide 1396; the nucleotide sequence of SEQ ID NO:4 from nucleotide 122 to nucleotide 1396; the nucleotide sequence of SEQ ID NO:6 from nucleotide 71 to nucleotide 1225; or the nucleotide sequence of SEQ ID NO:6 from nucleotide 11 to nucleotide 1225.

The invention also provides isolated polynucleotides comprising a nucleotide sequence encoding a peptide or protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:5;

(b) the amino acid sequence of SEQ ID NO:5 from amino acids 41 to 425;

(c) the amino acid sequence of SEQ ID NO:7;

(d) the amino acid sequence of SEQ ID NO:7 from amino acids 24 to 408; and

(e) fragments of (a)-(d) having a biological activity of the U4 hematopoietin receptor superfamily chain. Other preferred embodiments encode the amino acid sequence of SEQ ID NO:5; the amino acid sequence of SEQ ID NO:5 from amino acids 41 to 425; the amino acid sequence of SEQ ID NO:7; and the amino acid sequence of SEQ ID NO:7 from amino acids 24 to 408.

Host cells, preferably mammalian cells, transformed with the polynucleotides are also provided.

In other embodiments, the invention provides a process for producing a U4 protein. The process comprises:

(a) growing a culture of the host cell of the present invention in a suitable culture medium; and

(b) purifying the human U4 protein from the culture.

Proteins produced according to these methods are also provided.

The present invention also provides for an isolated U4 protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:5;

(b) the amino acid sequence of SEQ ID NO:5 from amino acids 41 to 425;

(c) the amino acid sequence of SEQ ID NO:7;

(d) the amino acid sequence of SEQ ID NO:7 from amino acids 24 to 408; and

(e) fragments of (a)-(d) having a biological activity of the U4 hematopoietin receptor superfamily chain.

Preferably the protein comprises the amino acid sequence of SEQ ID NO:5; the amino acid sequence of SEQ ID NO:5 from amino acids 41 to 425; the amino acid sequence of SEQ ID NO:7; or the amino acid sequence of SEQ ID NO:7 from amino acids 24 to 408. In other preferred embodiments, the specified amino acid sequence is part of a fusion protein (with an additional amino acid sequence not derived from U4). Preferred fusion proteins comprise an antibody fragment, such as an Fc fragment.

Pharmaceutical compositions comprising a protein of the present invention and a pharmaceutically acceptable carrier are also provided.

The present invention further provides for compositions comprising an antibody which specifically reacts with a protein of the present invention.

Detailed Description of Preferred Embodiments

The inventors of the present application have for the first time identified and provided polynucleotides encoding the U4 hematopoietin receptor superfamily chain (hereinafter "U4"or "U4 protein"), including without limitation polynucleotides encoding murine and human U4.

A 79 amino acid region of the human IL-5 receptor (LMTNAFISIIDDLSKYDVQVRAAVSSMCREAGLWSEWSQPIYVGND EHKPLREWFVIVIMATICFILLIL, SEQ ID NO:1) was used to search the GenBank EST database using the TBLASTN algorithm. EST W66776 was identified with homology to this region, suggesting that this might encode a novel hematopoietin receptor. Translation of the reverse-complement of this EST using the GCG map program revealed a protein sequence in the second reading frame that contained the conserved WSXWS motif found in hematopoietin receptors. However, a stop codon was also present in this reading frame at nucleotide 227, indicating that this EST either was not a novel hematopoietin receptor, or that the DNA sequence in the EST was incorrect.

To determine whether this EST sequence might be related to a hematopoietin receptor, we screened a murine embryo library with an oligonucleotide probe of the sequence CTTGGCTTGG AAGAGGAAAT CCTTGAGAGC (SEQ ID NO:2). A full-length cDNA clone U6-3(1A) was identified and complete sequence was obtained. The DNA sequence and the predicted amino acid sequence for the murine protein are reported as SEQ ID NO:4 and SEQ ID NO:5, respectively. The murine protein encodes a novel member of the hematopoietin receptor family. It has a leader sequence, and the conserved cysteine pairs, PP, and WSXWS motifs characteristic of this family. This clone has no transmembrane or cytoplasmic domains. Alignment of this clone with the EST in GenBank revealed that the EST did have a frame shift mutation.

SEQ ID NO:4 provides the nucleotide sequence of a cDNA encoding the murine U4. SEQ ID NO:5 provides predicted the amino acid sequence of the receptor chain, including a putative signal sequence from amino acids 1-40. The mature murine U4 is believed to have the sequence of amino acids 41-383 of SEQ ID NO:5.

To identify additional related sequences in GenBank, the W66776 sequence was used to search GenBank using the BLASTN algorithm. A closely related EST, H14009, derived from human genomic DNA was identified. An oligonucleotide derived from this EST CTGAGCGTGC GCTGGGTGTC GCCAC (SEQ ID NO:3) was then used to isolate a cDNA clone from a human cDNA library. A cDNA clone (HU4-3B) encoding a full-length mature protein homolog was completely sequenced. This clone does not have a complete signal sequence, but does encode the entire predicted full-length mature protein. The human clone is 85% homologous at the DNA level with the mouse clone. The predicted amino acid sequences have 95% identity between human and mouse. The nucleotide and amino acid sequence for human U4 are reported as SEQ ID NO:6 and SEQ ID NO:7, respectively.

SEQ ID NO:6 provides the nucleotide sequence of a cDNA encoding the human U4. SEQ ID NO:7 provides predicted the amino acid sequence of the receptor chain, including a putative signal sequence from amino acids 1-23. The mature human U4 is believed to have the sequence of amino acids 24-380 of SEQ ID NO:7.

The murine and human clones were deposited with the American Type Culture Collection on January ___, 1997, as accession numbers ATCC _____ and ATCC _____, respectively.

Human U4 protein can be expressed by replacing the human leader sequence with the sequence of the murine leader, or by extending the human leader sequence with amino acids 1-14 of the murine sequence (MPAGRPGPVA QSAR, SEQ ID NO:8). Additionally, a longer cDNA or genomic clone encoding the actual human leader can be isolated using the sequences disclosed herein as probes.

Any forms of U4 proteins of less than full length are encompassed within the present invention and are referred to herein collectively with full length and mature forms as "U4" or "U4 proteins." U4 proteins of less than full length may be produced by expressing a corresponding fragment of the polynucleotide encoding the full-length U4 protein (SEQ ID NO:4 or SEQ ID NO:6). These corresponding polynucleotide fragments are also part of the present invention.

Modified polynucleotides as described above may be made by standard molecular biology techniques, including construction of appropriate desired deletion mutants, site-directed mutagenesis methods or by the polymerase chain reaction using appropriate oligonucleotide primers.

For the purposes of the present invention, a protein has "a biological activity of the U4 hematopoietin receptor superfamily chain" if it possess one or more of the biological activities of the corresponding mature U4 protein.

U4 or active fragments thereof (U4 proteins) may be fused to carrier molecules such as immunoglobulins. For example, soluble forms of the U4 may be fused through "linker" sequences to the Fc portion of an immunoglobulin. Other fusions proteins, such as those with GST, Lex-A or MBP, may also be used.

The invention also encompasses allelic variants of the nucleotide sequences as set forth in SEQ ID NO:4 or SEQ ID NO:6, that is, naturally-occurring alternative forms of the isolated polynucleotide of SEQ ID NO:4 or SEQ ID NO:6 which also encode U4 proteins, preferably those proteins having a biological activity of U4. Also included in the invention are isolated polynucleotides which hybridize to the nucleotide sequence set forth in SEQ ID NO:4 or SEQ ID NO:6 under highly stringent conditions (for example, 0.1xSSC at 65°C). Isolated polynucleotides which encode U4 proteins but which differ from the nucleotide sequence set forth in SEQ ID NO:4 or SEQ ID NO:6 by virtue of the degeneracy of the genetic code are also encompassed by the present invention. Variations in the nucleotide sequence as set forth in SEQ ID NO:4 or SEQ ID NO:6 which are caused by point mutations or by induced modifications are also included in the invention.

The present invention also provides polynucleotides encoding homologues of the murine and human U4 from other animal species, particularly other mammalian species. Species homologues can be identified and isolated by making probes or primers from the murine or human sequences disclosed herein and screening a library from an appropriate species, such as for example libraries constructed from PBMCs, thymus or testis of the relevant species.

The isolated polynucleotides of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman et al., Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the U4 protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means enzymatically or chemically ligated to form a covalent bond between the isolated polynucleotide of the invention and the expression control sequence, in such a way that the U4 protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

A number of types of cells may act as suitable host cells for expression of the U4 protein. Any cell type capable of expressing functional U4 protein may be used. Suitable mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK, Rat2, BaF3, 32D, FDCP-1, PC12, M1x or C2C12 cells.

The U4 protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. Soluble forms of the U4 protein may also be produced in insect cells using appropriate isolated polynucleotides as described above.

Alternatively, the U4 protein may be produced in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Suitable yeast strains include Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins.

Expression in bacteria may result in formation of inclusion bodies incorporating the recombinant protein. Thus, refolding of the recombinant protein may be required in order to produce active or more active material. Several methods for obtaining correctly folded heterologous proteins from bacterial inclusion bodies are known in the art. These methods generally involve solubilizing the protein from the inclusion bodies, then denaturing the protein completely using a chaotropic agent. When cysteine residues are present in the primary amino acid sequence of the protein, it is often necessary to accomplish the refolding in an environment which allows correct formation of disulfide bonds (a redox system). General methods of refolding are disclosed in Kohno, Meth. Enzym., 185:187-195 (1990). EP 0433225 and copending application USSN 08/163,877 describe other appropriate methods.

The U4 protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a polynucleotide sequence encoding the U4 protein.

The U4 protein of the invention may be prepared by growing a culture transformed host cells under culture conditions necessary to express the desired protein. The resulting expressed protein may then be purified from the culture medium or cell extracts. Soluble forms of the U4 protein of the invention can be purified from conditioned media. Membrane-bound forms of U4 protein of the invention can be purified by preparing a total membrane fraction from the expressing cell and extracting the membranes with a non-ionic detergent such as Triton X-100.

The U4 protein can be purified using methods known to those skilled in the art. For example, the U4 protein of the invention can be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) or polyetheyleneimine (PEI) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred (e.g., S-Sepharose® columns). The purification of the U4 protein from culture supernatant may also include one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; or by hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or by immunoaffinity chromatography. Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the U4 protein. Affinity columns including antibodies to the U4 protein can also be used in purification in accordance with known methods. Some or all of the foregoing purification steps, in various combinations or with other known methods, can also be employed to provide a substantially purified isolated recombinant protein. Preferably, the isolated U4 protein is purified so that it is substantially free of other mammalian proteins.

U4 proteins of the invention may also be used to screen for agents which are capable of binding to U4. Binding assays using a desired binding protein, immobilized or not, are well known in the art and may be used for this purpose using the U4 protein of the invention. Purified cell based or protein based (cell free) screening assays may be used to identify such agents. For example, U4

protein may be immobilized in purified form on a carrier and binding or potential ligands to purified U4 protein may be measured.

U4 proteins, purified from cells or recombinantly produced, may be used as a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may contain, in addition to U4 or inhibitor and carrier, various diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration.

The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-14, IL-15, G-CSF, stem cell factor, and erythropoietin. The pharmaceutical composition may also include anti-cytokine antibodies. The pharmaceutical composition may contain thrombolytic or anti-thrombotic factors such as plasminogen activator and Factor VIII. The pharmaceutical composition may further contain other anti-inflammatory agents. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with isolated U4 protein, or to minimize side effects caused by the isolated U4 protein. Conversely, isolated U4 protein may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

The pharmaceutical composition of the invention may be in the form of a liposome in which isolated U4 protein is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers which in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides,

lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, e.g., amelioration of symptoms of, healing of, or increase in rate of healing of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of isolated U4 protein is administered to a mammal. Isolated U4 protein may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, U4 protein may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering U4 protein in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of U4 protein used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of U4 protein is administered orally, U4 protein will be in the form of a tablet, capsule, powder, solution or elixir.

When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% U4 protein, and preferably from about 25 to 90% U4 protein. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of U4 protein, and preferably from about 1 to 50% U4 protein.

When a therapeutically effective amount of U4 protein is administered by intravenous, cutaneous or subcutaneous injection, U4 protein will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to U4 protein an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additive known to those of skill in the art.

The amount of U4 protein in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of U4 protein with which to treat each individual patient. Initially, the attending physician will administer low doses of U4 protein and observe the patient's response. Larger doses of U4 protein may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not generally increased further. It is contemplated that

the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.1 µg to about 100 mg of U4 protein per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the U4 protein will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

The polynucleotide and proteins of the present invention are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

Cytokine and Cell Proliferation/Differentiation Activity

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986;

Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., J. Immunol. 149:3778-3783, 1992; Bowman et al., J. Immunol. 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A.M. and Shevach, E.M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human Interferon γ, Schreiber, R.D. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6 - Nordan, R. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark, S.C. and Turner, K.J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

Immune Stimulating or Suppressing Activity

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, *i.e.*, in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present invention.

Using the proteins of the invention it may also be possible to immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as , for example, B7)), *e.g.*, preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (*e.g.*, B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins *in vivo* as described in Lenschow *et al.*, Science *257*:789-792 (1992) and Turka *et al.*, Proc. Natl. Acad. Sci USA, *89*:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function *in vivo* on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the

activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/*lpr/lpr* mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells *in vitro* with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the *in vitro* activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells *in vivo*.

In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (*e.g.*, sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present

invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected *ex vivo* with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection *in vivo*.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (*e.g.*, a cytoplasmic-domain truncated portion) of an MHC class I α chain protein and β_2 microglobulin protein or an MHC class II α chain protein and an MHC class II β chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (*e.g.*, B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA

78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowmanet al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: *In vitro* antibody production, Mond, J.J. and Brunswick, M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

Hematopoiesis Regulating Activity

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al.,

Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initiating cell assay, Sutherland, H.J. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

Research Uses and Utilities

polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays

(such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used in assay to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E.F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S.L. and A.R. Kimmel eds., 1987.

Nutritional Uses

Polynucleotides and proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the protein or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

U4 proteins of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the U4

protein and which may inhibit binding of ligands to the receptor. Such antibodies may be obtained using the entire U4 as an immunogen, or by using fragments of U4. Smaller fragments of the U4 may also be used to immunize animals. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Additional peptide immunogens may be generated by replacing tyrosine residues with sulfated tyrosine residues. Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J.Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, et al., FEBS Lett. 211, 10 (1987).

Neutralizing or non-neutralizing antibodies (preferably monoclonal antibodies) binding to U4 protein may also be useful therapeutics for certain tumors and also in the treatment of conditions described above. These neutralizing monoclonal antibodies may be capable of blocking ligand binding to the U4 receptor chain.

Example

Expression of U4 Protein

DNA encoding the full-length murine U4 protein was fused to a spacer sequence encoding Gly-Ser-Gly by PCR and ligated in frame with sequences encoding the hinge CH2 CH3 regions of human IgG1 in the COS-1 expression vector pED.Fc . The DNA was transfected into Cos cells and expression of the fusion protein was detected by ELISA using antibodies that detected the IgG1 portion of the protein. This demonstrated that the protein could be expressed and secreted in Cos cells.

All patent and literature references cited herein are incorporated by reference as if fully set forth.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: GENETICS INSTITUTE, INC.

(ii) TITLE OF INVENTION: CYTOKINE RECEPTOR CHAIN

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Genetics Institute, Inc.
(B) STREET: 87 CambridgePark Drive
(C) CITY: Cambridge
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02140

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Brown, Scott A.
(B) REGISTRATION NUMBER: 32,724
(C) REFERENCE/DOCKET NUMBER: GI5287-PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617) 498-8224
(B) TELEFAX: (617) 876-5851

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Leu Met Thr Asn Ala Phe Ile Ser Ile Ile Asp Asp Leu Ser Lys Tyr
1               5                  10                  15

Asp Val Gln Val Arg Ala Ala Val Ser Ser Met Cys Arg Glu Ala Gly
            20                  25                  30

Leu Trp Ser Glu Trp Ser Gln Pro Ile Tyr Val Gly Asn Asp Glu His
        35                  40                  45

Lys Pro Leu Arg Glu Trp Phe Val Ile Val Ile Met Ala Thr Ile Cys
    50                  55                  60
```

Phe Ile Leu Leu Ile Leu
65 70

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "probe"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CTTGGCTTGG AAGAGGAAAT CCTTGAGAGC 30

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "probe"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTGAGCGTGC GCTGGGTGTC GCCAC 25

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1656 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTCGACCTTC GCTGTCCGCG CCCAGTGACG CGCGTGAGGA CCCGAGCCCC AATCTGCACC 60

CCGCAGACTC GCCCCCGCCC CATACCGGCG TTGCAGTCAC CGCCCGTTGC GCGCCACCCC 120

CATGCCCGCG GGTCGCCCGG GCCCCGTCGC CCAATCCGCG CGGCGGCCGC CGCGGCCGCT 180

GTCCTCGCTG TGGTCGCCTC TGTTGCTCTG TGTCCTCGGG GTGCCTCGGG GCGGATCGGG 240

AGCCCACACA GCTGTAATCA GCCCCCAGGA CCCCACCTTT CTCATCGGCT CCTCCCTGCA 300

AGCTACCTGC TCTATACATG GAGACACACC TGGGGCCACC GCTGAGGGGC TCTACTGGAC 360

CCTCAATGGT CGCCGCCTGC CCTCTGAGCT GTCCCGCCTC CTTAACACCT CCACCCTGGC 420

CCTGGCCCTG GCTAACCTTA ATGGGTCCAG GCAGCAGTCA GGAGACAATC TGGTGTGTCA 480

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CGCCCGAGAT GGCAGCATTC TGGCTGGCTC CTGCCTCTAT GTTGGCTTGC CCCCTGAGAA      540

GCCTTTTAAC ATCAGCTGCT GGTCCCGGAA CATGAAGGAT CTCACGTGCC GCTGGACACC      600

GGGTGCACAC GGGGAGACAT TCTTACATAC CAACTACTCC CTCAAGTACA AGCTGAGGTG      660

GTACGGTCAG GATAACACAT GTGAGGAGTA CCACACTGTG GGCCCTCACT CATGCCATAT      720

CCCCAAGGAC CTGGCCCTCT TCACTCCCTA TGAGATCTGG GTGGAAGCCA CCAATCGCCT      780

AGGCTCAGCA AGATCTGATG TCCTCACACT GGATGTCCTG GACGTGGTGA CCACGGACCC      840

CCCACCCGAC GTGCACGTGA GCCGCGTTGG GGGCCTGGAG GACCAGCTGA GTGTGCGCTG      900

GGTCTCACCA CCAGCTCTCA AGGATTTCCT CTTCCAAGCC AAGTACCAGA TCCGCTACCG      960

CGTGGAGGAC AGCGTGGACT GGAAGGTGGT GGATGACGTC AGCAACCAGA CCTCCTGCCG     1020

TCTCGCGGGC CTGAAGCCCG GCACCGTTTA CTTCGTCCAA GTGCGTTGTA ACCCATTCGG     1080

GATCTATGGG TCGAAAAAGG CGGGAATCTG GAGCGAGTGG AGCCACCCCA CCGCTGCCTC     1140

CACCCCTCGA AGTGAGCGCC CGGGCCCGGG CGGCGGGGTG TGCGAGCCGC GGGGCGGCGA     1200

GCCCAGCTCG GGCCCGGTGC GGCGCGAGCT CAAGCAGTTC CTCGGCTGGC TCAAGAAGCA     1260

CGCATACTGC TCGAACCTTA GTTTCCGCCT GTACGACCAG TGGCGTGCTT GGATGCAGAA     1320

GTCACACAAG ACCCGAAACC AGGACGAGGG GATCCTGCCC TCGGGCAGAC GGGGTGCGGC     1380

GAGAGGTCCT GCCGGCTAAA CTCTAAGGAT AGGCCATCCT CCTGCTGGGT CAGACCTGGA     1440

GGCTCACCTG AATTGGAGCC CCTCTGTACC ATCTGGGCAA CAAAGAAACC TACCAGAGGC     1500

TGGGGCACAA TGAGCTCCCA CAACCACAGC TTTGGTCCAC ATGATGGTCA CACTTGGATA     1560

TACCCCAGTG TGGGTAGGGT TGGGGTATTG CAGGGCCTCC CAAGAGTCTC TTTAAATAAA     1620

TAAAGGAGTT GTTCAGGTCC CGAAAAAAAA GTCGAC                                1656
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 425 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Pro Ala Gly Arg Pro Gly Pro Val Ala Gln Ser Ala Arg Arg Pro
1               5                  10                  15

Pro Arg Pro Leu Ser Ser Leu Trp Ser Pro Leu Leu Leu Cys Val Leu
            20                  25                  30

Gly Val Pro Arg Gly Gly Ser Gly Ala His Thr Ala Val Ile Ser Pro
        35                  40                  45

Gln Asp Pro Thr Phe Leu Ile Gly Ser Ser Leu Gln Ala Thr Cys Ser
    50                  55                  60

Ile His Gly Asp Thr Pro Gly Ala Thr Ala Glu Gly Leu Tyr Trp Thr
65                  70                  75                  80
```

```
Leu Asn Gly Arg Arg Leu Pro Ser Glu Leu Ser Arg Leu Leu Asn Thr
                85                  90                  95

Ser Thr Leu Ala Leu Ala Leu Ala Asn Leu Asn Gly Ser Arg Gln Gln
            100                 105                 110

Ser Gly Asp Asn Leu Val Cys His Ala Arg Asp Gly Ser Ile Leu Ala
        115                 120                 125

Gly Ser Cys Leu Tyr Val Gly Leu Pro Pro Glu Lys Pro Phe Asn Ile
    130                 135                 140

Ser Cys Trp Ser Arg Asn Met Lys Asp Leu Thr Cys Arg Trp Thr Pro
145                 150                 155                 160

Gly Ala His Gly Glu Thr Phe Leu His Thr Asn Tyr Ser Leu Lys Tyr
                165                 170                 175

Lys Leu Arg Trp Tyr Gly Gln Asp Asn Thr Cys Glu Glu Tyr His Thr
            180                 185                 190

Val Gly Pro His Ser Cys His Ile Pro Lys Asp Leu Ala Leu Phe Thr
        195                 200                 205

Pro Tyr Glu Ile Trp Val Glu Ala Thr Asn Arg Leu Gly Ser Ala Arg
    210                 215                 220

Ser Asp Val Leu Thr Leu Asp Val Leu Asp Val Val Thr Thr Asp Pro
225                 230                 235                 240

Pro Pro Asp Val His Val Ser Arg Val Gly Gly Leu Glu Asp Gln Leu
                245                 250                 255

Ser Val Arg Trp Val Ser Pro Pro Ala Leu Lys Asp Phe Leu Phe Gln
            260                 265                 270

Ala Lys Tyr Gln Ile Arg Tyr Arg Val Glu Asp Ser Val Asp Trp Lys
        275                 280                 285

Val Val Asp Asp Val Ser Asn Gln Thr Ser Cys Arg Leu Ala Gly Leu
    290                 295                 300

Lys Pro Gly Thr Val Tyr Phe Val Gln Val Arg Cys Asn Pro Phe Gly
305                 310                 315                 320

Ile Tyr Gly Ser Lys Lys Ala Gly Ile Trp Ser Glu Trp Ser His Pro
                325                 330                 335

Thr Ala Ala Ser Thr Pro Arg Ser Glu Arg Pro Gly Pro Gly Gly Gly
            340                 345                 350

Val Cys Glu Pro Arg Gly Gly Glu Pro Ser Ser Gly Pro Val Arg Arg
        355                 360                 365

Glu Leu Lys Gln Phe Leu Gly Trp Leu Lys Lys His Ala Tyr Cys Ser
    370                 375                 380

Asn Leu Ser Phe Arg Leu Tyr Asp Gln Trp Arg Ala Trp Met Gln Lys
385                 390                 395                 400

Ser His Lys Thr Arg Asn Gln Asp Glu Gly Ile Leu Pro Ser Gly Arg
                405                 410                 415

Arg Gly Ala Ala Arg Gly Pro Ala Gly
            420                 425
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1579 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GCGGCCGCCG CCGTTGCTGC CCCTGCTGCT GCTGCTCTGC GTCCTCGGGG CGCCGCGAGC 60

CGGATCAGGA GCCCACACAG CTGTGATCAG TCCCCAGGAT CCCACGCTTC TCATCGGCTC 120

CTCCCTGCTG GCCACCTGCT CAGTGCACGG AGACCCACCA GGAGCCACCG CCGAGGGCCT 180

CTACTGGACC CTCAACGGGC GCCGCCTGCC CCCTGAGCTC TCCCGTGTAC TCAACGCCTC 240

CACCTTGGCT CTGGCCCTGG CCAACCTCAA TGGGTCCAGG CAGCGGTCGG GGGACAACCT 300

CGTGTGCCAC GCCCGTGACG GCAGCATCCT GGCTGGCTCC TGCCTCTATG TTGGCCTGCC 360

CCCAGAGAAA CCCGTCAACA TCAGCTGCTG GTCCAAGAAC ATGAAGGACT TGACCTGCCG 420

CTGGACGCCA GGGGCCCACG GGGAGACCTT CCTCCACACC AACTACTCCC TCAAGTACAA 480

GCTTAGGTGG TATGGCCAGG ACAACACATG TGAGGAGTAC CACACAGTGG GGCCCCACTC 540

CTGCCACATC CCCAAGGACC TGGCTCTCTT TACGCCCTAT GAGATCTGGG TGGAGGCCAC 600

CAACCGCCTG GGCTCTGCCC GCTCCGATGT ACTCACGCTG GATATCCTGG ATGTGGTGAC 660

CACGGACCCC CCGCCCGACG TGCACGTGAG CCGCGTCGGG GGCCTGGAGG ACCAGCTGAG 720

CGTGCGCTGG GTGTCGCCAC CCGCCCTCAA GGATTTCCTC TTTCAAGCCA AATACCAGAT 780

CCGCTACCGA GTGGAGGACA GTGTGGACTG GAAGGTGGTG GACGATGTGA GCAACCAGAC 840

CTCCTGCCGC CTGGCCGGCC TGAAACCCGG CACCGTGTAC TTCGTGCAAG TGCGCTGCAA 900

CCCCTTTGGC ATCTATGGCT CCAAGAAAGC CGGGATCTGG AGTGAGTGGA GCCACCCCAC 960

AGCCGCCTCC ACTCCCCGCA GTGAGCGCCC GGGCCCGGGC GGCGGGGCGT GCGAACCGCG 1020

GGGCGGAGAG CCGAGCTCGG GGCCGGTGCG GCGCGAGCTC AAGCAGTTCC TGGGCTGGCT 1080

CAAGAAGCAC GCGTACTGCT CCAACCTCAG CTTCCGCCTC TACGACCAGT GGCGAGCCTG 1140

GATGCAGAAG TCGCACAAGA CCCGCAACCA GGACGAGGGG ATCCTGCCCT CGGGCAGACG 1200

GGGCACGGCG AGAGGTCCTG CCAGATAAGC TGTAGGGGCT CAGGCCACCC TCCCTGCCAC 1260

GTGGAGACGC AGAGGCCGAA CCCAAACTGG GGCCACCTCT GTACCCTCAC TTCAGGGCAC 1320

CTGAGCCACC CTCAGCAGGA GCTGGGGTGG CCCCTGAGCT CCAACGGCCA TAACAGCTCT 1380

GACTCCCACG TGAGGCCACC TTTGGGTGCA CCCCAGTGGG TGTGTGTGTG TGTGTGAGGG 1440

TTGGTTGAGT TGCCTAGAAC CCCTGCCAGG GCTGGGGGTG AGAAGGGGAG TCATTACTCC 1500

CCATTACCTA GGGCCCCTCC AAAAGAGTCC TTTTAAATAA ATGAGCTATT TAGGTGCTGT 1560

GAAAAAAAAA AAAAAAAAA 1579

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 408 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Arg Pro Pro Pro Leu Leu Pro Leu Leu Leu Leu Leu Cys Val Leu Gly
1               5                  10                  15

Ala Pro Arg Ala Gly Ser Gly Ala His Thr Ala Val Ile Ser Pro Gln
            20                  25                  30

Asp Pro Thr Leu Leu Ile Gly Ser Ser Leu Leu Ala Thr Cys Ser Val
        35                  40                  45

His Gly Asp Pro Pro Gly Ala Thr Ala Glu Gly Leu Tyr Trp Thr Leu
    50                  55                  60

Asn Gly Arg Arg Leu Pro Pro Glu Leu Ser Arg Val Leu Asn Ala Ser
65                  70                  75                  80

Thr Leu Ala Leu Ala Leu Ala Asn Leu Asn Gly Ser Arg Gln Arg Ser
                85                  90                  95

Gly Asp Asn Leu Val Cys His Ala Arg Asp Gly Ser Ile Leu Ala Gly
            100                 105                 110

Ser Cys Leu Tyr Val Gly Leu Pro Pro Glu Lys Pro Val Asn Ile Ser
        115                 120                 125

Cys Trp Ser Lys Asn Met Lys Asp Leu Thr Cys Arg Trp Thr Pro Gly
    130                 135                 140

Ala His Gly Glu Thr Phe Leu His Thr Asn Tyr Ser Leu Lys Tyr Lys
145                 150                 155                 160

Leu Arg Trp Tyr Gly Gln Asp Asn Thr Cys Glu Glu Tyr His Thr Val
                165                 170                 175

Gly Pro His Ser Cys His Ile Pro Lys Asp Leu Ala Leu Phe Thr Pro
            180                 185                 190

Tyr Glu Ile Trp Val Glu Ala Thr Asn Arg Leu Gly Ser Ala Arg Ser
        195                 200                 205

Asp Val Leu Thr Leu Asp Ile Leu Asp Val Val Thr Thr Asp Pro Pro
    210                 215                 220

Pro Asp Val His Val Ser Arg Val Gly Gly Leu Glu Asp Gln Leu Ser
225                 230                 235                 240

Val Arg Trp Val Ser Pro Pro Ala Leu Lys Asp Phe Leu Phe Gln Ala
                245                 250                 255

Lys Tyr Gln Ile Arg Tyr Arg Val Glu Asp Ser Val Asp Trp Lys Val
            260                 265                 270

Val Asp Asp Val Ser Asn Gln Thr Ser Cys Arg Leu Ala Gly Leu Lys
        275                 280                 285

Pro Gly Thr Val Tyr Phe Val Gln Val Arg Cys Asn Pro Phe Gly Ile
    290                 295                 300
```

```
Tyr Gly Ser Lys Lys Ala Gly Ile Trp Ser Glu Trp Ser His Pro Thr
305                 310                 315                 320

Ala Ala Ser Thr Pro Arg Ser Glu Arg Pro Gly Pro Gly Gly Gly Ala
                325                 330                 335

Cys Glu Pro Arg Gly Gly Glu Pro Ser Ser Gly Pro Val Arg Arg Glu
            340                 345                 350

Leu Lys Gln Phe Leu Gly Trp Leu Lys Lys His Ala Tyr Cys Ser Asn
        355                 360                 365

Leu Ser Phe Arg Leu Tyr Asp Gln Trp Arg Ala Trp Met Gln Lys Ser
    370                 375                 380

His Lys Thr Arg Asn Gln Asp Glu Gly Ile Leu Pro Ser Gly Arg Arg
385                 390                 395                 400

Gly Thr Ala Arg Gly Pro Ala Arg
                405
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Pro Ala Gly Arg Pro Gly Pro Val Ala Asn Ser Ala Arg
1               5                   10
```

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence of SEQ ID NO:4 from nucleotide 242 to nucleotide 1396;

(b) the nucleotide sequence of SEQ ID NO:6 from nucleotide 71 to nucleotide 1225;

(c) a nucleotide sequence varying from the sequence of the nucleotide sequence specified in (a) or (b) as a result of degeneracy of the genetic code;

(d) a nucleotide sequence capable of hybridizing under stringent conditions to the nucleotide specified in (a) or (b);

(e) a nucleotide sequence encoding a species homologue of the sequence specified in (a) or (b); and

(f) an allelic variant of the nucleotide sequence specified in (a) or (b).

2. The polynucleotide of claim 1 wherein said nucleotide sequence encodes for a protein having a biological activity of the U4 hematopoietin receptor superfamily chain.

3. The polynucleotide of claim 1 wherein said nucleotide sequence is operably linked to an expression control sequence.

4. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:4 from nucleotide 122 to nucleotide 1396.

5. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:6 from nucleotide 11 to nucleotide 1225.

6. A host cell transformed with the polynucleotide of claim 3.

7. The host cell of claim 6, wherein said cell is a mammalian cell.

8. A process for producing a U4 protein, said process comprising:

(a) growing a culture of the host cell of claim 6 in a suitable culture medium; and

(b) purifying the U4 protein from the culture.

9. An isolated U4 protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:5;

(b) the amino acid sequence of SEQ ID NO:5 from amino acids 41 to 425;

(c) the amino acid sequence of SEQ ID NO:7;

(d) the amino acid sequence of SEQ ID NO:7 from amino acids 24 to 408; and

(e) fragments of (a)-(d) having a biological activity of the U4 hematopoietin receptor superfamily chain.

10. The protein of claim 9 comprising the amino acid sequence of SEQ ID NO:5.

11. The protein of claim 9 comprising the sequence from amino acid 41 to 425 of SEQ ID NO:5.

12. The protein of claim 9 comprising the amino acid sequence of SEQ ID NO:7.

13. The protein of claim 9 comprising the sequence from amino acid 24 to 408 of SEQ ID NO:7.

14. A pharmaceutical composition comprising a protein of claim 9 and a pharmaceutically acceptable carrier.

15. A protein produced according to the process of claim 8.

16. A composition comprising an antibody which specifically reacts with a protein of claim 9.

17. An isolated polynucleotide comprising a nucleotide sequence encoding a peptide or protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:5;

(b) the amino acid sequence of SEQ ID NO:5 from amino acids 41 to 425;

(c) the amino acid sequence of SEQ ID NO:7;

(d) the amino acid sequence of SEQ ID NO:7 from amino acids 24 to 408; and

(e) fragments of (a)-(d) having a biological activity of the U4 hematopoietin receptor superfamily chain.

18. The protein of claim 9 wherein said amino acid sequence is part of a fusion protein.

19. The protein of claim 18 comprising an Fc fragment.

INTERNATIONAL SEARCH REPORT

Inter ...nal Application No
PCT/US 98/00334

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/19 C07K14/715 A61K38/17 C07K16/18 C12N15/62

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARRA M ET AL.: "AC: AA049280. Mus musculus cDNA clone 479043 5' end similar to SW: IL6B_MOUSE Q00560 interleukin-6 receptor beta chain precursor." EMBL SEQUENCE DATABASE, 31 December 1996, HEIDELBERG, GERMANY, XP002062032 see the whole document ---	1,3,6-8, 15
X	MARRA M ET AL.: "AC: W66776. Mus musculus cDNA clone 387741 5' end similar to PIR:B38252 granulocyte colony-stimulating factor receptor precursor." EMBL SEQUENCE DATABASE, 15 June 1996, HEIDELBERG, GERMANY, XP002062033 cited in the application see the whole document ---	1,3,6-8, 15

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
16 April 1998	29/04/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Oderwald, H

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter ial Application No
PCT/US 98/00334

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	LI Q Y: "AC: H14009. EST00035 Homo sapiens genomic clone D2-17 5'." EMBL SEQUENCE DATABASE , 3 July 1995, HEIDELBERG, GERMANY, XP002062036 cited in the application see the whole document ---	1,3,6-8, 15
X	HILLIER L ET AL.: "AC: W46604. Homo sapiens cDNA clone 324067 3'." EMBL SEQUENCE DATABASE, 28 May 1996, HEODELBERG, GERMANY, XP002062037 see the whole document ---	1,3,6-8, 15
A	WO 96 08510 A (PROGENITOR INC) 21 March 1996 see abstract; figure 1; example 6 see page 2, line 32 - page 3, line 30 ---	
E	WO 98 11225 A (NICOLA NICOS ANTONY ;FABRI LOUIS (AU); FARLEY ALISON (AU); NASH AN) 19 March 1998 see the whole document -----	1,3,6-8, 15

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter ional Application No
PCT/US 98/00334

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9608510 A	21-03-96	US 5643748 A	01-07-97
		AU 3419495 A	29-03-96
		CA 2176463 A	21-03-96
		EP 0730606 A	11-09-96
WO 9811225 A	19-03-98	NONE	